RELEASE OF ALLELOCHEMICALS BY THREE TROPICAL SPONGES (DEMOSPONGIAE) AND THEIR TOXIC EFFECTS ON CORAL SUBSTRATE COMPETITORS

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Three sponge species (*Xestospongia*, *Acervochalina*, *Plakortis* spp.) from Mactan I., Philippines, were shown to release allelochemicals directly into the water. These allelochemicals were demonstrated to be toxic to one or more scleractinian coral species (*Acropora*, *Pocillopora*, *Porites* spp.) and, for one of the sponges tested, to one hydrozoan coral (*Millepora* sp.). The five coral species tested (including *Montipora*) were both numerically and spatially dominant organisms at the study site. Toxicity tests involved exposing corals brought into the laboratory to water that had been conditioned by the sponges. Responses of each coral species to each sponge allelochemical varied. The allelochemical from *Acervochalina* was found to be highly toxic (51-75% tissue death) to both *Pocillopora* and *Acropora*, and had only a moderate effect (26-50% tissue death) on *Porites*. Allelochemicals of *Xestospongia* and *Plakortis* were moderately and weakly toxic (11-25% tissue death) to *Millepora*, respectively. Neither sponge was toxic towards the other coral species. *Montipora* was not affected by allelochemicals from any of the sponges. Dead coral was noted in many positions around the sponges in the field, but mainly in the direction of the current. This might support, although not confirm, an allelopathic effect. The influence of allelochemicals on the small scale and large scale spatial structuring of coral reefs is discussed. ❐ *Porifera, corals, allelochemical, coral reef, toxicity, chemical ecology, Philippines, Xestospongia, Acervochalina, Plakortis.*

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Release of allelochemicals into the surrounding water, as a defense against benthic spatial competitors, fouling organisms, or microorganisms, has been demonstrated in several groups of marine organisms (see Bakus et al., 1986). These organisms include sponges (Thompson, 1985; Walker et al., 1985; Porter & Targett, 1988; Targett, 1988; Bingham & Young, 1991), soft corals (Coll & Sammarco, 1983; Sammarco et al., 1983, 1985; La Barre et al.., 1986; Maida et al., 1995), an anemone (Bak & Borsboom, 1984), possibly an alga (Littler & Littler, 1997) and hard corals (Fearon, 1997; Koh, 1997). Although only few studies have yet been undertaken, allelochemicals are believed to play a role in structuring particular marine habitats (Jackson & Buss, 1975; Davis et al., 1989; Turon et al., 1996; Thacker et al., 1998), whereas the magnitude of this role is still uncertain.

La Barre et al. (1986) demonstrated that when three species of soft corals were relocated in pairs under contact and non-contact conditions, tissue necrosis was observed in all contact pairs. Only one species of soft coral produced tissue necrosis when placed near, but not in contact with, the other two species of soft coral. They suggested that avoidance behavior, such as those caused by allelopathy, may contribute to the dispersion patterns of plants and animals in space. In another study on soft corals, Sammarco et al. (1985) found that scleractinian corals varied in their susceptibility when exposed to soft corals in both contact and non-contact conditions.

Porter & Targett (1988) demonstrated that the sponge *Plakortis halichondroides* was capable of damaging or destroying tissue in all coral species examined, with almost half the corals growing naturally in contact with, or near to, these sponges experiencing bleaching or tissue necrosis. It was suggested that by creating dead zones on the corals *P. halichondroides* was subsequently able to overgrow them. In a more recent study, Turon et al. (1996) suggested that *Crambe crambe* could have an impact on adjacent organisms by possibly releasing allelochemicals

into the surrounding water. The allelochemical effect of this sponge was at the small scale level (centimeters) (Turon et al., 1996), where patterns such as an increase in the amount of dead coral or tolerant species found adjacent to the producer of allelochemicals became evident. Turon et al. (1996) suggested that at different scales different patterns might be recognised. On previous trips to the Philippines we observed similar bleached areas or dead zones up to 1cm in width in areas where some of the species of sponges came in contact, or close contact, with coral species.

The purpose of the present study was to: 1) Determine if three tropical sponges were releasing allelochemicals potentially toxic to five hard corals on a coral reef; 2) Identify patterns due to allelochemical effects, such as the presence or absence of dead space adjacent to sponges (given that sponges releasing toxic allelochemicals would have a preponderance for dead coral, or tolerant species adjacent to them, and these might be predominantly located in the direction where the allelochemicals were most concentrated), and 3) Determine if the allelochemicals were toxic to common substrate competitors.

MATERIALS AND METHODS

Our study site was located on a limestone reef approximately 0.25km off the Tambuli Resort on Mactan I., Philippines. The study site was chosen on the basis of its high marine diversity and close proximity to the Maribago Marine Station, operated by the University of San Carlos. Depths in the vicinity of the study site ranged from 5m, where a seagrass bed began, to 15m, which bordered the start of a steep slope. However, most of the experiments conducted in this study were located between 8-11m depth. The study was conducted in May and June, 1996. (For a detailed description and map of the site see Bakus & Nishiyama, 1999, this volume). Species of sponges included: *Xestospongia* sp. (Haplosclerida: Petrosiidae), *Acervochalina* sp. (Haplosclerida: Chalinidae), and *Plakortis* sp. (Homosclerophorida: Plakinidae), and the corals: *Acropora* sp. (Scleractinia: Acroporidae), *Millepora* sp. (Milleporina: Milleporidae), *Montipora* sp. (Scleractinia: Acroporidae), *Pocillopora* sp. (Scleractinia: Pocilloporidae) and *Porites* sp. (Scleractinia: Poritidae).

ALLELOCHEMICAL DETECTION AND ISOLATION. To determine if sponges were releasing chemicals into the water, an allelochemical collecting apparatus was constructed from a battery operated bilge pump and SEP paks (Fig. 1). This apparatus was a modified version of one used by Coll et al. (1982), and later by Schulte et al. (1991). The battery operated bilge pump was fitted at its outflow hose with a step-down tubing connector (2cm to 0.7cm), which allowed two plastic screw valves to be connected (diameter 0.7cm). C18 SEP paks were initially conditioned by passing 10ml of Etoh followed by 10ml of deionised water through each SEP pak using a plastic pipette. Conditioning SEP paks before use was critical, otherwise flow would be

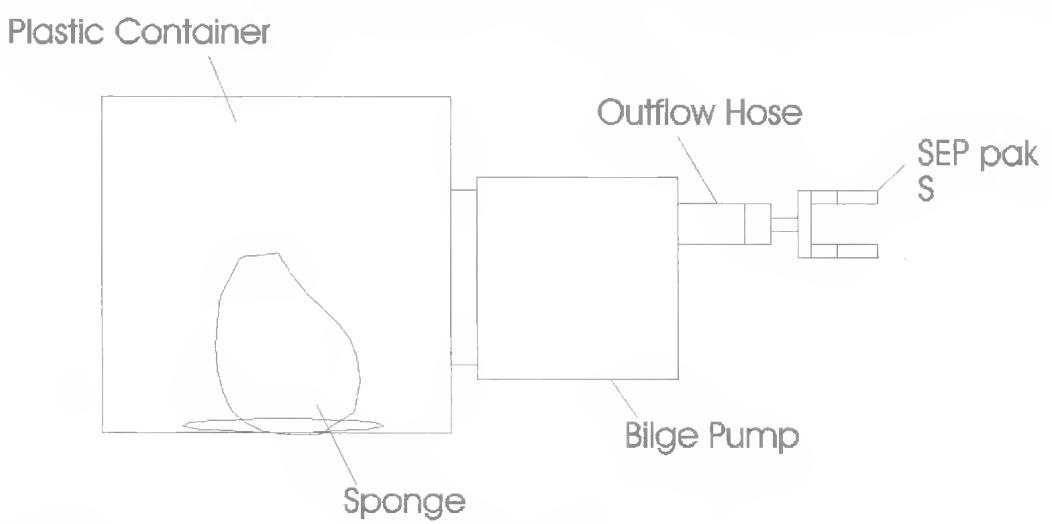

FIG. 1. Allelochemical isolating apparatus used to isolate allelochemicals from sponges.

interrupted. *In situ*, one conditioned SEP pak was inserted into each end of the plastic screw valve. At the inflow end of the pump, a plastic collapsible container (20x30x50cm) was attached which had a large hole cut at the bottom (diameters 20x30cm; Fig. 1). The purpose of the container was to assist in concentrating allelochemicals to increase probability of detection. Once the SEP paks were attached, the apparatus was placed over the sponge, with the sponge protruding into, but not touching, the plastic container. Although flow rate was determined both at sea level and 10m depth by allowing the output water to flow into an empty container for 30mins, no flow meter continuously measured flow rates in this initial model. Water surrounding eight unidentified sponges was sampled for 1.5hrs, coinciding with the maximum continuous run-time for the pumps. A control apparatus was set-up approximately 0.5m upstream from each apparatus covering a sponge. A newer model is currently being constructed with a flow meter and an added battery included for longer run times and continuous monitoring. Eight different sponge species were sampled at the study site using this apparatus to detect the release of any allelochemicals. After each run, the SEP paks and the whole sponge specimens being sampled were immediately sealed separately in small plastic bags, and upon arrival to the laboratory (not more than 20mins later), were placed in a freezer (-5°C). Upon departure from the Philippines the SEP paks and sponge specimens were placed in dry ice, and upon arrival in the USA the items were placed into a deep freezer (-20°C). Chemicals were initially eluted out by passing 20ml of dichloromethane, followed by 20ml of Etoh through SEP paks. Extractions were air evaporated for approximately 24hrs. It was subsequently discovered that extraction with 50ml acetone produced higher extraction yields. Thereafter, only acetone extractions were used. Thin layer chromatographies (TLC) were conducted using acetone as the mobile phase throughout the extraction process to insure that chemicals were not lost during the evaporation . TLCs were visualised using both long and short UV light. There was no noticeable changes in chemical composition of the extract within this period. TLCs were run on SEP pak extracts, control SEP paks, and whole sponge extracts (5gm samples of each sponge species extracted in acetone).

SMALL SCALE PATTERNS AROUND SPONGES. Patterns of organisms found adjacent to, or within approximately 5cm from, sponges were investigated by first taking photographs of between 29-39 individuals of each species and their adjacent organisms *in situ* with a Nikonos V camera. A scale bar with a waterproof compass attached to it was laid parallel to the direction of the current and placed next to each sponge before photographs were taken. Sponges in each photograph were divided into four quadrants and the presence or absence of dead coral in each quadrant was recorded. The widths of the dead zones on corals ranged from 1-10mm. Seven categories were devised to quantify the positions of dead coral: 1) Horizontal: dead coral only on two quadrants that faced currents (roughly in the E and W positions); 2)Vertical: dead coral in two quadrants perpendicular to the current (roughly in the N and S directions); 3) Dead coral found more in horizontal than vertical positions; 4) Half-half: dead coral found equally in horizontal and vertical positions; 5) More dead coral in vertical than horizontal positions; 6) Dead coral found completely around the sponge; and 7) Only live organisms completely surrounding the sponge.

TOXICITY OF ALLELOCHEMICALS ON HARD CORALS. Fifty pieces (approximately 3-4cm long) from each of two individuals of the five coral species were obtained at the study site using a geological pick, and placed in separate plastic bags for each coral species. These plastic bags were then placed into buckets to ensure minimal mechanical stress during transport. Sponges were removed from the substrate by chiselling around each sponge and carefully removing them. Sponges were then positioned and left on dead coral for one week to allow recuperation following their removal. When ready for use, two whole sponges of each of the three sponge species were placed in separate plastic bags. Care was taken to minimise damage when removing and transporting corals and sponges. Both were returned to the laboratory and sponges were immediately placed in a 1L beaker filled with filtered seawater obtained from the study site (ambient water, gravity filtered with a #1 Whatman filter). Corals were placed in separate plastic buckets with unfiltered seawater from the site until ready for use. Sponges were allowed to condition the water for 1hr before the water was passed through a Whatman #1 filter and gravity filtered. This filtration process required less than 30mins. Two sponge individuals from each species were allowed to condition water separately to test for individual variability

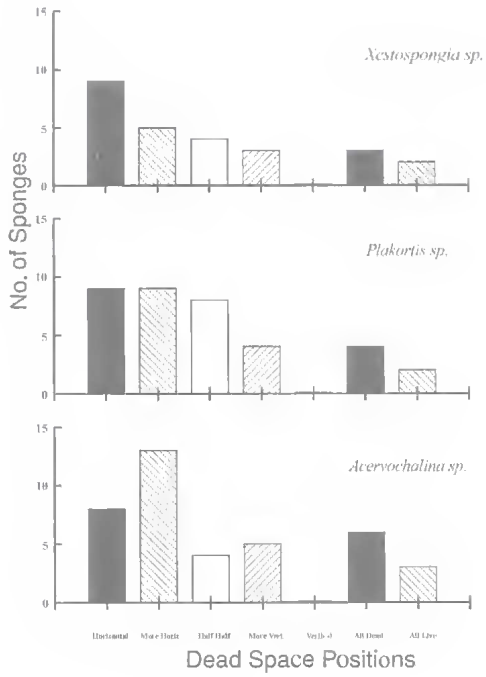

FIG. 2. Positions of dead space found around sponges from Mactan Island, Philippines. (See text for detailed descriptions of categories).

towards allelochemical toxicity. While this filtration process was carried out, corals were placed in glass finger bowls along with 100ml of ambient water. When sponge-conditioned water was ready, water in finger bowls was decanted off and replaced with an equal volume of sponge-conditioned water. A control treatment of 1L of non-conditioned, filtered seawater was also initially set aside for 1hr. The condition of corals and water temperature were noted. Corals were exposed to conditioned and control water experiments for 24hrs. A change in temperature from 25°C to 22°C was recorded for the water in the bowls. The ambient water temperature at the study site during the experiment was 27°C. After this 24hr period, corals were returned to their original site of collection and placed under a metal cage (5x30x100cm; mesh size approx. 1cm). The condition of coral fragments was monitored for 4 days thereafter. Following this period, close-up photographs were taken, both *in situ* and after the corals were returned to the laboratory. These photographs were enlarged (approximately 200%), and the area of the exposed surface of each piece of coral, as well as the area of dead tissue, traced onto acetate. The percentage of dead tissue was determined after estimating the total area of each coral fragment and the area of dead tissue, using an image analysis program (SigmaScan [SPSS Inc], 1998). Detailed notes and sketches of each piece of coral *in situ* were made earlier and were compared to the calculated dead tissue areas. In all instances both estimates were comparable. Coral tissue was considered to be dead or dying if there were signs of cell death or, as in most cases, actual detachment of tissue from the skeletal base. Loss of coloration was also observed in all dead tissue. One-way ANOVA and post hoc Tukey tests were conducted on the percentage tissue death of each coral species, with the allelochemical and control treatments being the main factor.

RESULTS

Of the eight unidentified sponges sampled for allelochemical release at our study site, three were shown to be releasing allelochemicals. These species were subsequently identified as *Acervochalina*, *Plakortis*, and *Xestospongia* spp. TLC comparisons between water immediately surrounding these three sponges, control water samples, and whole sponge extracts confirmed that allelochemicals were found within the respective sponges but not in the water column upstream from the sponges.

The presence and position of dead space adjacent to the the species of sponges occurred predominantly in the direction facing (either partially or completely) the current flow (Fig. 2). Since the direction of water flow was reversed periodically, depending on whether the tide was rising or falling, dead space occurred on both sides of the sponge facing these currents. In *Xestospongia* dead space occurred in horizontal positions (i.e. facing currents) and positions mostly facing current flows in 71% of specimens (Fig. 2). Dead space was located in these positions in 73% of *Plakortis*, and 66% of *Acervochalina*. In no instance for any of the three species of sponges was dead space found only in the vertical position (i.e. not facing current flow).

Water conditioned individually by each of the three species of sponges was toxic to one or more of the five coral species tested (Fig. 3). Responses of each coral species towards each sponge

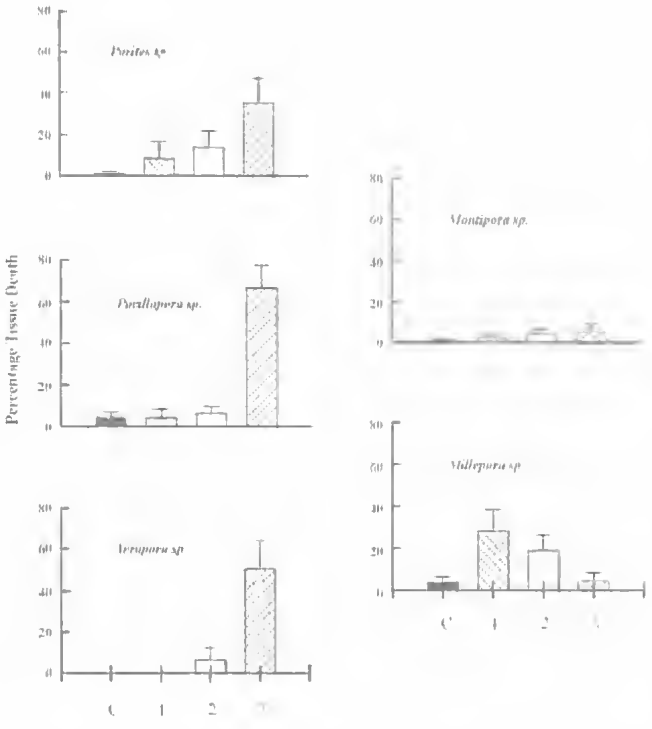

FIG 3. Average percentage tissue death of five coral species exposed to water conditioned by three sponge species (Key: 1=*Xestospongia*; 2=*Plakortis*; 3=*Acervochalina*; C= control treatments, representing corals exposed to filtered seawater). (Bar represents 1 S.E.).

allelochemical varied. Control corals experienced minimal tissue death, but not always less death than all the other treatments (Fig. 3). For all coral species, except *Montipora*, significant differences existed among the treatments (one-way ANOVA). Results of the Tukey tests and average percentage coral tissue death show that when compared to controls, the allelochemical of *Acervochalina* was highly toxic (51-75% tissue death) to *Pocillopora* ($P<0.05$) and *Acropora* ($P<0.05$), and had a moderate effect (26-50% tissue death) towards *Porites* ($P<0.05$) The sponge was not toxic towards *Millepora*. *Xestospongia* and *Plakortis* were moderately (26-50% tissue death; $P<0.05$) and weakly toxic (11-25% tissue death) ($P=0.05$) to *Millepora*, respectively. Neither sponge was toxic towards the other coral species. *Montipora* was not affected by allelochemicals from any of the sponges ($P>0.05$).

DISCUSSION

Results show that filtered seawater conditioned by all three species of sponges, *Xestospongia*, *Plakortis* and *Acervochalina*, were toxic to at least one species of hard coral (*Porites*, *Pocillopora*, *Acropora* and *Millepora*). Responses of corals towards sponge-conditioned water varied, as expected. Susceptibility of corals in contact with, or in proximity to sponges suggests the possibility that sponge allelochemicals may influence patterns of distributions of organisms adjacent to sponges. In other words, certain tolerant species of corals may grow adjacent to sponges, whereas others may never or rarely be found in close proximity.

In a parallel study we conducted at our study site, whole sponges were transplanted and placed into direct contact with corals. We determined that several species of corals were highly susceptible to (i.e. damaged by) several species of sponges. Under natural conditions, these corals were rarely found growing in direct contact with these sponges (Nishiyama & Bakus, unpublished information). In rare cases where they did grow naturally adjacent to these sponges, a zone of dead or bleached tissue was often noted at the site of contact. Furthermore, if a species of sponge was not found to be deleterious to a species of coral, incidences of growth with direct contact between the two species were more often noted. This corresponds to field observations made by Porter & Targett (1988) where almost half the corals growing next to *Plakortis halichondroides* experienced bleaching or tissue necrosis. Both these studies support our hypothesis that water borne allelochemicals may deter particular substrate competitors from growing in direct contact with sponges. It should be cautioned, however, that laboratory bioassays using sponge-conditioned water were conducted on corals in still water, whereas in nature currents probably have a major influence in dissipating allelochemicals, and thus, their physiological impact may not be as extensive as those observed in the laboratory.

Although particular sponges may have an impact on adult corals, these toxic effects may also have a greater impact in preventing coral larvae settling adjacent to sponges. The occurrence of dead coral space around sponges, mainly in the direction facing currents, may reflect the directions that highest allelochemical concentrations occur given that toxins are carried away from sponges. Although only suggestive, this supports the notion that toxic allelochemicals were being released by sponges. Maida et al. (1995) provided evidence to suggest that a soft coral could influence the direction from which recruitment occurred. An alternative hypothesis is that some hydrodynamic effect prevents settlement of larvae in areas facing the current. It may also be advantageous for a sponge to encourage adjacent settlement and growth of coral species which are susceptible to the sponges' allelochemicals. This would ensure that the sponge would be able to grow and expand into the adjacent area.

Through their release of allelochemicals sponges may stop the growth of adjacent, adult substrate competitors, such as corals. However, this effect may operate only on a local or small scale, at least for the three species of sponges investigated here, because zones of dead or bleached tissue on corals growing adjacent to the three species of sponges extended for only 1cm at most. *Acervochalina*, however, may also possibly overgrow corals, with an observed high density in comparison to the other two species, generally having the highest toxicity towards corals, and being thinly encrusting with possibly greater lateral growth. *Acervochalina* was also observed growing on branches of corals that were dead at the bases (where the sponges occupied), yet alive at the tips (where the sponge had not yet extended). Allelochemicals of *Acervochalina* may operate to stop corals from overgrowing it and as a mechanism to kill coral tissue, to open up space for its own growth.

In a study conducted by Bakus & Nishiyama (1999, this volume), data from transects at the study site show that no apparent sequence existed where a particular substratum type (i.e. live hard coral, sponges, coral rubble, etc.) was found next to sponges; that is, the succession of organisms and substrata were independent of each other. In that study, however, individual species of both corals and sponges were not differentiated, and therefore, specific species pairs may exist. Using transect line data techniques, Turon et al. (1996) investigated the possibility of allelochemicals being released by sponges. They determined that *Crambe crambe* had toxic effects up to 1cm from particular coral substrate competitors, corresponding to the effective distance of allelochemicals suggested in the present study. Turon et al. (1996) also suggested that these small scale effects might only be detected by sampling at a small scale (centimeters), whereas sampling at 3cm intervals produced a different outcome. Where allelochemicals have effective distances at a scale of less than 1cm, observations made at 1cm intervals may not suffice in detecting these chemical interactions. In the present study, extreme care was taken to accurately determine dead space and organisms adjacent to sponges, and in most cases, measurements were determined to the nearest millimeter.

The chemical nature of allelochemicals released by sponges, as measured by SEP paks, are currently being ascertained, as are the toxicities of these isolated chemicals towards the five species of corals. Although SEP paks retained chemicals released by sponges, this does not necessarily confirm any toxicity by the sponge. Only isolation of the active chemicals from sponges and verification of their toxicity towards corals would confirm that these are allelochemicals deleterious to corals. Data from another study conducted by the authors are currently being analyzed (Nishiyama & Bakus, unpublished data), involving the deterrence of settlement of substrate competitors by sponges placed next to plates kept in the water at the site for approximately one month.

After hard corals, sponges were the dominant organisms at Mactan I. (Bakus & Nishiyama, 1999, this volume), also showing relatively high diversity (Bakus & Nishiyama, unpublished data). Of eight sponges investigated, three released allelochemicals into the water column, suggesting that many more sponges, not investigated here, may release allelochemicals. Thus, sponge allelochemicals may play an important role in structuring the coral reef community at a small scale, local level. More work is needed, however, to determine the extent of this role.

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